Encapsulation of Stem Cells in Core-Shell Hydrogel Microcapsules of Various Stiffness using Microfluidics

THESIS

Presented in Partial Fulfillment of the Requirements for Honors Research with Distinction in the Undergraduate School of The Ohio State University

By

Peter Anthony Bielecki

Undergraduate in Biomedical Engineering

The Ohio State University

2013

Thesis Committee:

Dr. Xiaoming He, Advisor

Dr. Mark Ruegsegger

Abstract

Stem cell based therapies serve as a potential option to treat a variety of organ dysfunctions such as Parkinson's, Alzheimer's, cardiovascular, and musculoskeletal diseases because stem cells can be coaxed to differentiate into many types of more specialized cells. However, it is challenging to control the niches including bio-chemical and mechanical factors that regulate the proliferation and differentiation of stem cells. One potential way to resolve this challenge is to encapsulate stem cells in 3D biocompatible matrices of various core compositions. In this project, we successfully encapsulated human embryonic palatal (HEPM) stem cells in microcapsules with a 3D collagen core matrix and alginate shell using a novel multilayer microfluidic chip. This was done by creating a device that shears cell suspension and alginate flow using mineral oil flow infused with calcium ion to solidify alginate. Studies were conducted to illustrate how stiffness and fiber geometry of the collagen core matrix affect cell viability and proliferation in the core-shell hydrogel microcapsules. These stem cell laden microcapsules could be very useful for cell-based therapy and regenerative medicine applications.

Acknowledgments

This work was partially supported by grants from NSF (CBET-1033426) and NIH (R01EB012108). The author would also like to thank graduate student Pranay Agarwal for his project collaboration and advising, the Ohio State Undergraduate Honors Committee in the College of Engineering for their support towards the research proposal, and summer intern Joe Jeffery for his time spent in the laboratory on this project.

Vita

May 2009 .....Lakeview High School

2009 to present .....B.S. Biomedical Engineering, The Ohio State University

2011 to present.....Student Employee, Clinical Engineering Department, Wexner Medical Center

Fields of Study

Major Field: Biomedical Engineering

Table of Contents

List of Figures

Chapter 1: Background

Stem cells encapsulated in a microcapsule composed of natural polymers like alginate can serve as a potential option in cell based therapies to treat a variety of organ dysfunctions such as osteoarthritis, Parkinson's, Alzheimer's, and cardiovascular diseases [1]. Encapsulated stem cells can differentiate into desired cell types like osteoblasts, neurons or cardiac cells to aid in rebuilding diseased bone, brain, and heart tissues, respectively. It has been hypothesized that differentiated stem cells have the potential to speed the recovery of the tissues or organs [2]. However, differentiation of stem cells into a desired cell type is not straightforward. There are various factors collectively called the "stem cell niche" that determines the fate of stem cells, including soluble chemicals and cell-cell and cell-matrix interactions [2,3]. These interactions, however, can be modulated by regulating the size and stiffness of alginate microcapsules. For instance, stem cells encapsulated in microcapsules composed of a stiff matrix are reported to differentiate into osteocytes [4]. Therefore, controlling the size and stiffness of microcapsules is very important in managing the differentiation of stem cells into a desired cell type.

Chapter 2: Project Introduction

With today's obesity epidemic, aging baby boomer population, and increased life expectancy, a more effective treatment is needed to increase the quality of life of the already 27 million victims of osteoarthritis (OA) [5]. Current treatments for OA include invasive surgery and implantation, temporary over-the-counter pain relievers, and severe lifestyle changes that limit patient mobility [6]. Human embryonic palatal mesenchyme (HEPM) stem cells are osteoblast precursor cells that have potential to treat osteoimmunological bone disorders like OA due to their calcium and phosphorus deposition properties [7,8]. As mesenchymal stem cells differentiate based on the stiffness of their environment, HEPM stem cells best mimic *in-vivo* behavior when in contact with a matrix of a high modulus of elasticity [4].

Type I collagen is the most abundant extracellular matrix protein found in connective tissues. Collagen fibers form when monomers polymerize to form 3D structures by means of self assembly. The mechanical properties of collagen can vary with changing pH, collagen concentration, and ionic strength. By manipulating collagen concentrations from 0.3 mg/mL to 3.0 mg/mL, the stiffness of a matrix can vary from 1.5 to 24.3 kPa, respectively [9]. Because of this, varying collagen concentration is an

excellent method to alter matrix stiffness in order to analyze HEPM stem cell proliferation.

HEPM stem cell proliferation can be studied in varying collagen concentrations via either 2D or 3D cell culturing systems. Though 2D cell culturing is easily performed and cost effective, the unnatural environment can alter cell morphology, gene and protein expression, and cell proliferation and differentiation. 3D cell culturing is advantageous because it more closely recapitulates the *in-vivo* environment experienced by the HEPM stem cells.

In this project, a polydimethylsiloxane (PDMS) based microfluidic device was fabricated to produce core (collagen)-shell (alginate) microcapsules of various collagen concentrations by employing flow focusing principles. The core-shell microcapsules enable cells to be encapsulated in collagen matrices that closely resemble *in-vivo* environments compared to traditional techniques that encapsulate cells in a uniform alginate matrix. This device uses mineral oil infused with calcium chloride to shear both cell suspension (with collagen) and alginate flows, solidifying alginate in the process. HEPM stem cells were successfully encapsulated in these core-shell microcapsules of varying stiffness of 3D core matrix. Further, studies were conducted to illustrate how stiffness and fiber geometry of the collagen core matrix affect cell viability and proliferation in the microcapsules.

Chapter 3: Materials and Methods

Core-Shell Microfluidic Device Fabrication

To create the multilayer microfluidic device, a silicon master was fabricated using photo lithography at the Nanotech West facility at The Ohio State University. Photosensitive epoxy (SU-8 3050, Microchem) was spin-coated onto 5 inch wafers previously cleaned with nitrogen gas and etched for alignment ease before undergoing soft baking and pre-baking. The wafers were exposed to UV light through the shadow mask to cross-link the structure of the microchannels prior to post exposure baking. Because the microfluidic device is to be multilayer in support of maintaining desired fluid dynamics, the process of spin coating, baking, and exposure was repeated to create a deeper set of channels. The SU-8 patterns on the substrate were developed in SU-8 developer (Microchem) for 10 min, rinsed with IPA (isopropyl alcohol) and dried using nitrogen gas. Once this master was fabricated, PDMS was poured onto the silicon substrate and cured at 90 ^{o}C. The cured PDMS was peeled off, producing the pattern of microfluidic channels that was used in the experiment. The PDMS pattern was cleaned and treated with oxygen plasma for 40 seconds before being aligned under the microscope using methanol to slow the bonding process. The microfluidic device was heated at ~115 ^{o}C for 24 hours to ensure hydrophobicity.

Experimental Procedure

Syringe pumps were used to drive the injection of the continuous phase (mineral oil + Ca) and dispersed phases (alginate and HEPM stem cells + collagen) into the microfluidic system. A schematic of the flow-focusing microfluidic device is shown in **Figure 1** below. The solution of collagen and HEPM stem cells suspended in 0.3 M Mannitol + HEPES and 1% cellulose were flowed from the innermost inlet, 2% alginate in 0.3 M Mannitol + HEPES was flowed from the middle inlet, and emulsified mineral oil infused with calcium ions was flowed from the outermost inlet. The microfluidic device was linked to syringes via polytetrafluoroethylene (PTFE) tubing before the experiments commenced. The dispersed phase was sheared by the incoming continuous (oil) phase to create microcapsules from the differences in surface tensions while simultaneously gelling the alginate shell to leave the collagen + HEPM stem cell core. After running the experiment at 4 ^{o}C to ensure the fluidity of collagen, the microcapsules were exposed to room temperature to allow collagen to polymerize. The cells were then stained with calcein-am and ethidium homodimer to determine cell viability.

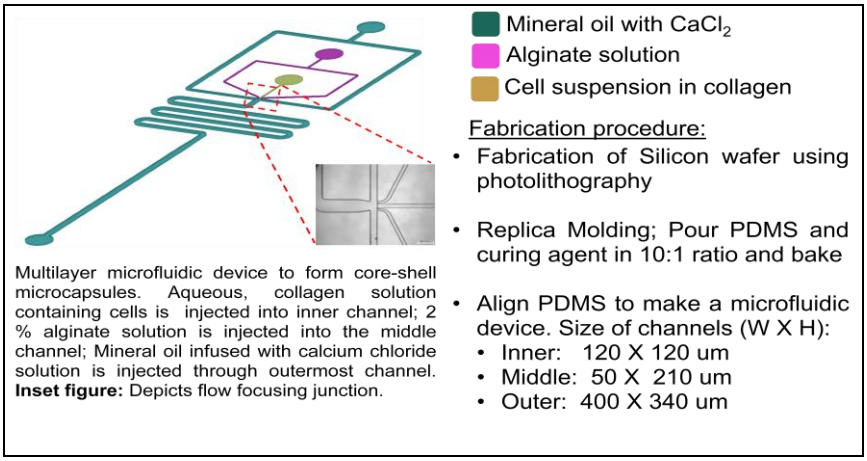
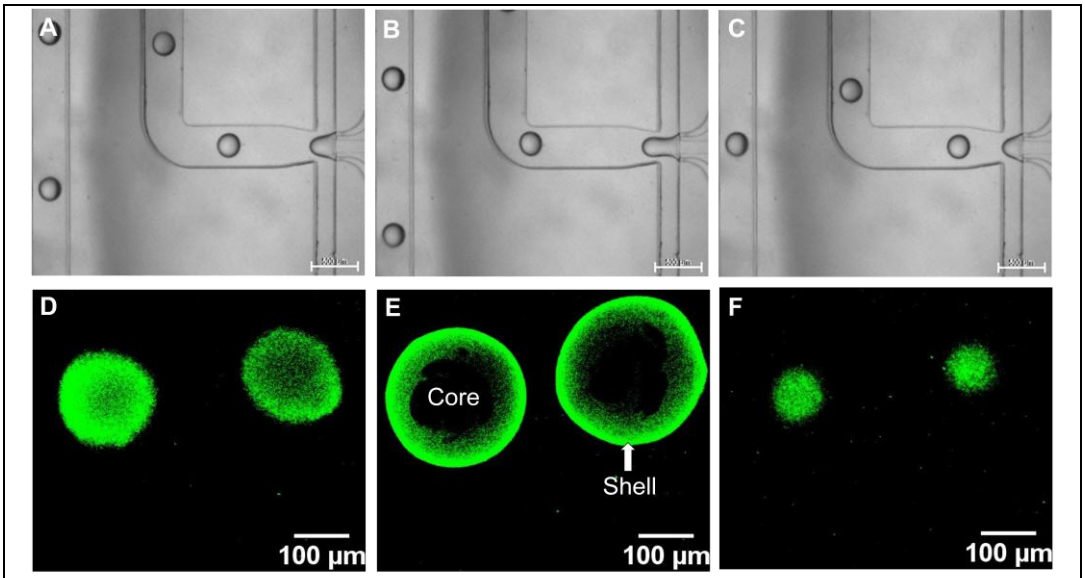


Figure 1: Schematic of flow-focusing, core-shell microfluidic device

Chapter 4: Results

Core-Shell Microcapsule Confirmation

Experiments were run to confirm the fabricated multilayer microfluidic device created core-shell microcapsules that have potential to hold biocompatible matrices in their cores. Mineral oil + calcium was flowed from the outermost inlet, 2% alginate solution stained with FITC labeled IgG (immunoglobulin G) was run through the middle inlet, and 1% cellulose solution was flowed from the innermost inlet. **Figure 2** below depicts a time lapse of microcapsule formation and a "z"-stack compilation of 10X fluorescent images confirming the creation of an alginate shell and cellulose core.

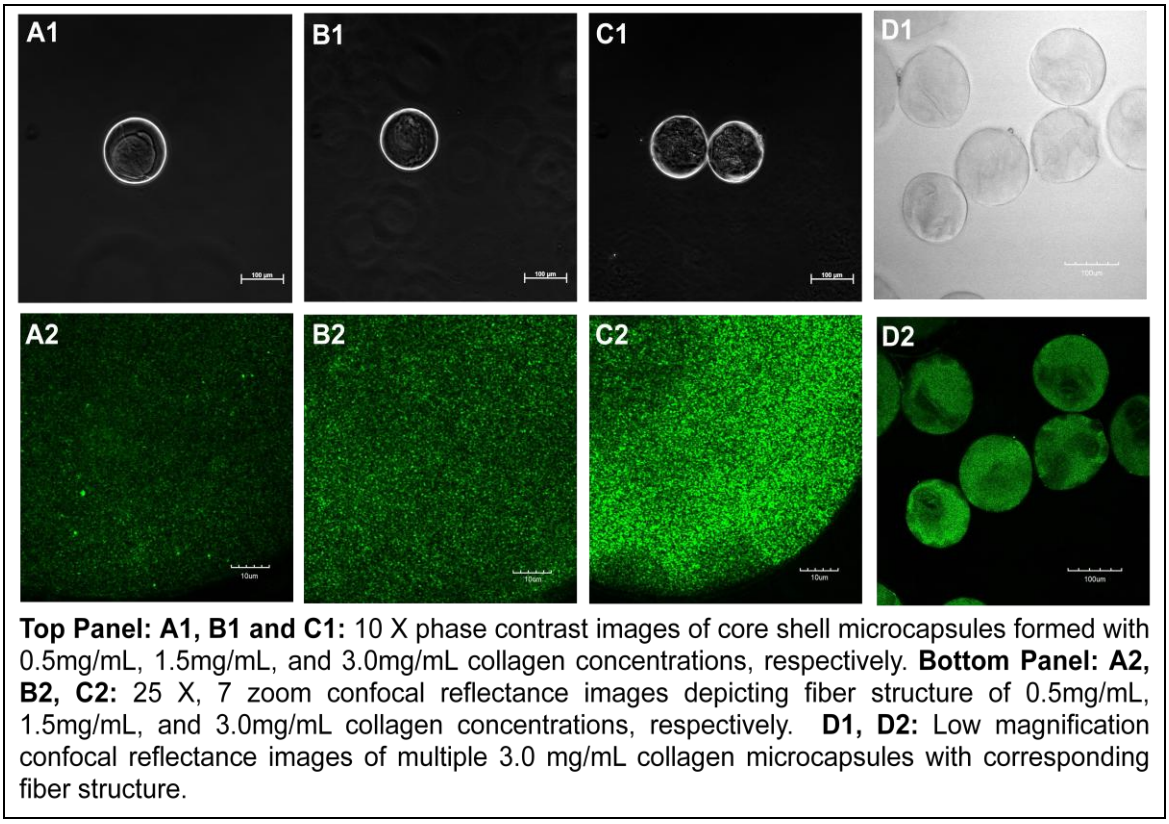


Top Panel: A, B and C: Time lapse (Bright field at 2.5 X) images showing the process of microcapsule formation in a microfluidic device. **Bottom panel: D, E, F:** 10X images of bottom, middle, and top planes of microcapsule stained with FITC IgG.

Figure 2: Core-shell microcapsule formation

Core-Shell Microcapsules of Varying Stiffness

Core-shell microcapsules of varying stiffness were created by flowing collagen concentrations of 0.5 mg/mL, 1.5 mg/mL, and 3.0 mg/mL into the innermost inlet (core) of the microfluidic device. **Figure 3** below compares the morphology of the microcapsule cores with core stiffness and depicts the confirmation of varying collagen fiber structure, shown in green, using confocal reflectance microscopy.



Top Panel: A1, B1 and C1: 10 X phase contrast images of core shell microcapsules formed with 0.5mg/mL, 1.5mg/mL, and 3.0mg/mL collagen concentrations, respectively. **Bottom Panel: A2, B2, C2:** 25 X, 7 zoom confocal reflectance images depicting fiber structure of 0.5mg/mL, 1.5mg/mL, and 3.0mg/mL collagen concentrations, respectively. **D1, D2:** Low magnification confocal reflectance images of multiple 3.0 mg/mL collagen microcapsules with corresponding fiber structure.

Figure 3: Varying collagen density/stiffness in microcapsule core

Control Culture of HEPM Stem Cells in 3D Collagen Matrix

A control experiment was conducted to observe the proliferation of HEPM stem cells in varying 3D collagen matrices. Films of 1% alginate and 1 mg/mL collagen were created on the bottom of a 24-well plate. Different cell suspensions were administered to the wells consisting of 0.5 mg/mL, 1.5 mg/mL, or 3.0 mg/mL collagen concentration, each holding a cell density of $2.5x10^6$ HEPM stem cells/mL. **Figure 4** below depicts the HEPM stem cells in varying 3D collagen matrices on day 0 and live(green)/dead(red) staining after 3 days of culture. The cells were highly viable and spread out presumably by attaching to the matrix of collagen at 1.5 and 3 mg/ml. Also, the matrices of higher collagen concentration began aggregating and were no longer homogenous after 3 days.

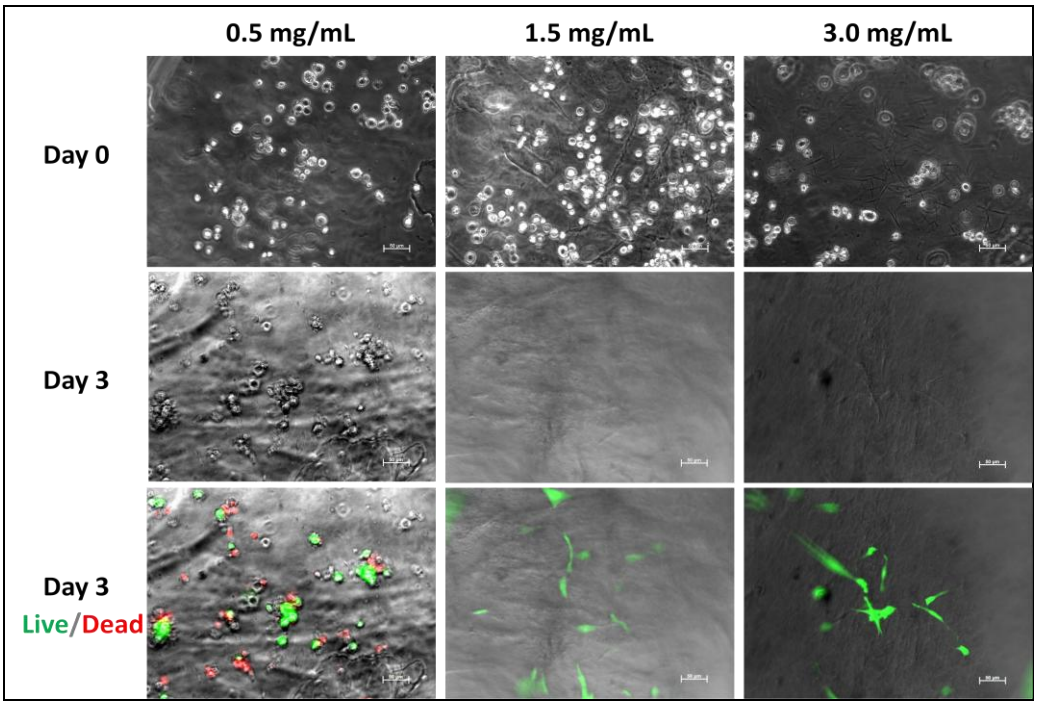


Figure 4: Control of HEPM stem cell attachment in varying 3D collagen matrices

Encapsulation of HEPM Stem Cells in 3D Collagen Cores

HEPM stem cells were encapsulated in 3D matrix cores with a collagen concentration of 3 mg/mL and cell concentration of $2.5x10^6$ HEPM stem cells/mL. Mineral oil + calcium was flowed at 4 mL/hr, 2% alginate solution was flowed at 220 μL/hr, and cell + collagen suspension was flown through a 120 μm wide channel at 150 μL/hr to produce core-shell microcapsules of < 200 μm diameter. **Figure 5** below depicts HEPM stem cell proliferation inside the 3D collagen core of the microcapsules after 1, 3, and 6 days of culture.

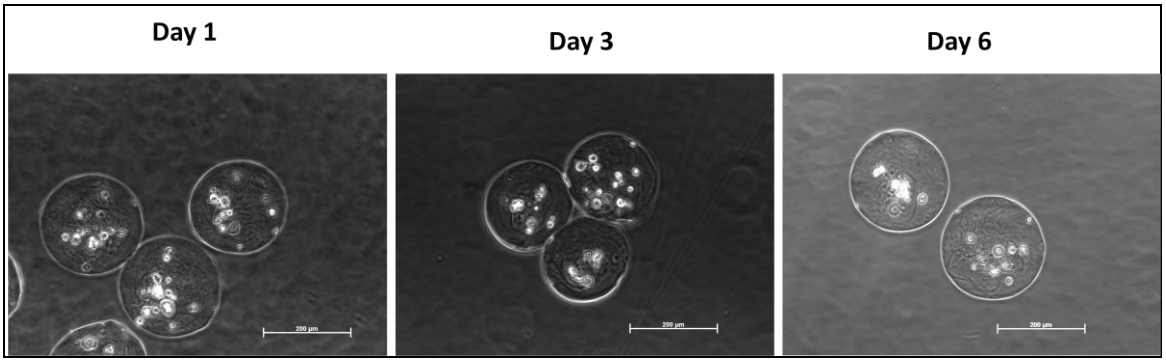


Figure 5: Culture of $2.5x10^6$ HEPM stem cells/mL in 3 mg/mL core-shell microcapsules of < 200 μm diameter

HEPM stem cells were then encapsulated in 3D matrix cores with a collagen concentration of 3 mg/mL and cell concentration of $2.0x10^6$ HEPM stem cells/mL. Mineral oil + calcium was flown at 2 mL/hr, 2% alginate solution was flown at 100 μL/hr, and cell + collagen suspension was flown through a 200 μm wide channel at 80 μL/hr to produce core-shell microcapsules of ~ 300 μm diameter. **Figure 6** below depicts

the HEPM stem cell proliferation inside the 3D collagen core of the microcapsules at day 0 and after 1 day of culture. Cell viability after encapsulation exceeded 90%.

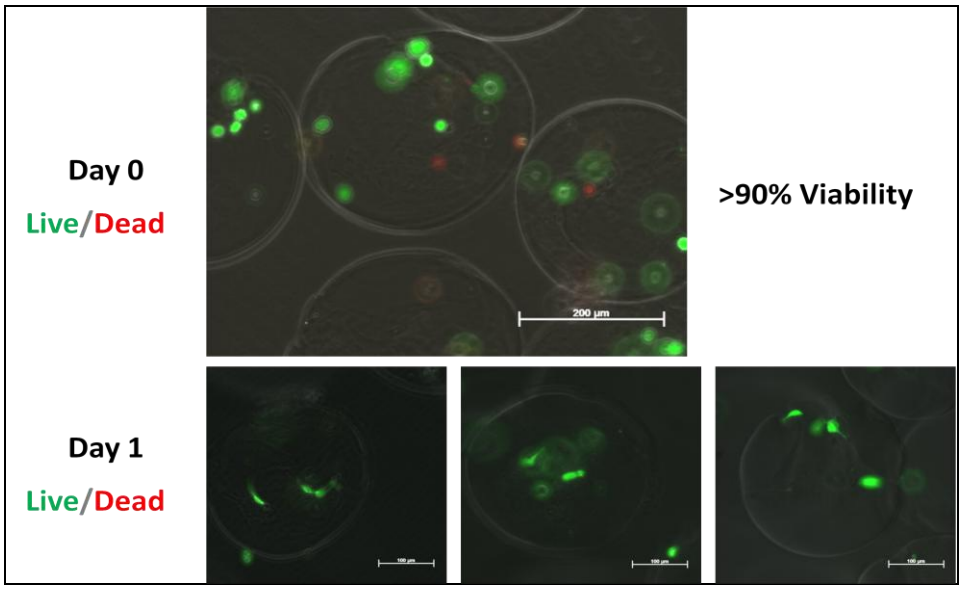


Figure 6: Culture of 2.0 x10^6 HEPM stem cells/mL in 3 mg/mL core-shell microcapsules of ~300 µm diameter

Chapter 5: Discussion

A multilayer, flow-focusing microfluidic device was fabricated that successfully creates core-shell microcapsules. The contents of these core-shell microcapsules can be substituted to create a wide range of biocompatible matrices for different cell types. In this study, microcapsules with cores of varying collagen density were created. It is evident that the core becomes less pronounced as collagen concentration increases. This is likely due to the increased diffusion of collagen into the alginate shell that occurs as a result of the delay between microcapsule collection in 4 ^{o}C and microcapsule exposure to room temperature. Confocal reflectance images confirm the increased density of collagen in the core as collagen concentration increases and, thus, modulus of elasticity increases to create a stiffer 3D matrix for prospective cells.

The extension of HEPM stem cells into their collagen environment was illustrated in a control experiment using a flat, 3D matrix. It is evident that cell structure is similar in all collagen matrices on day 0, while dark hashes of collagen fibers are more apparent in matrices of higher collagen concentrations. However, day 3 shows increased HEPM stem cell spreading in the stiffer 3 mg/mL collagen matrix compared to the 1.5 mg/mL matrix. HEPM stem cell spreading in the 0.5 mg/mL collagen matrix is virtually non-existent, with more cell death incurred, likely, to the low matrix stiffness. After 3 days, the higher collagen concentration matrices were no longer homogenous as they were at day 0.

Collagen aggregations formed as HEPM stem cells reached out into their environments to modify the matrix by drawing collagen fibers closer to accommodate proliferation and migration.

HEPM stem cells were encapsulated in 3 mg/mL collagen matrix cores of various sized microcapsules. Samples with cell concentrations of $2.5x10^6$ HEPM stem cells/mL inside the 3D collagen core of < 200 μm diameter microcapsules after 1, 3, and 6 days of culture showed no apparent proliferation. However, it was found that samples with cell concentrations of $2.0x10^6$ HEPM stem cells/mL began spreading and proliferating inside the 3D collagen core of ~ 300 μm diameter microcapsules after only 1 day. This is likely due to the increased space available for HEPM stem cell spreading and decreased competition for collagen. The amount of collagen in the microcapsule is finite, however, and the cells are not free to optimize their surrounding collagen concentration in support of spreading and proliferating as they were in the control experiment. Though the cell concentration was decreased, there likely exists some competition for collagen.

Future steps include increasing and optimizing microcapsule size and initial collagen concentration. This will allow for more cell spreading and proliferation and less competition in order for the HEPM stem cells to more closely mimic *in-vivo* behavior and function. Temperature control adjustments will be made to heat the collection as to minimize the amount of collagen diffusion into the alginate shell. Once the proliferation of HEPM stem cells in the core-shell microcapsules is optimized, differentiation studies will take place to develop osteoblast cell lines from the HEPM cells in the collagen matrix for hard bone regeneration to treat musculoskeletal diseases. Therefore, these stem

cell laden microcapsules show great promise for cell-based therapy and regenerative medicine applications.

References

1. *Alginate-based Encapsulation of Cells: Past, Present, and Future.* **Heiko Zimmermann, Stephen G. Shirley and Ulrich Zimmermann.** 2007, s.n., Current Diabetes Report, Vol. 7.

2. *Out of Eden: Stem Cells and Their Niches.* **Hogan, Fiona M. Watt and Brigid L. M.** 2000, Science, pp. 1427-1430.

3. *Microfabrication-based Modulation of Embryonic Stem Cell Differentiation.* **Jaesung Park, Cheul H. Cho , Natesh Parashurama , Yawen Li , François Berthiaume , Mehmet Toner , Arno W. Tilles and Martin L. Yarmush.** 2007, Lab on a Chip, Vol. 7, pp. 1018-1028.

4. *Growth Factors, Matrices, and Forces Combine and Control Stem Cells.* **Dennis E. Discher, David J. Mooney, Peter W. Zandstra.** 2009, Science, Vol. 324, pp. 1673-1677.

5. "Osteoarthritis and You." *Centers for Disease Control and Prevention*. CDC, Feb. 2012. Web. Mar. 2013.

6. A.D.A.M. Medical Encyclopedia. "Osteoarthritis." *PubMed Health*. U.S. National Library of Medicine, Jan. 20011. Web. Mar. 2013.

7. *Epigenetic regulation of osteogenesis: human embryonic palatal mesenchymal cells.* **Andre Barkhordarian, Jay Sison, Riana Cayabyab, Nicole Mahanian, Francesco Chiappelli.** 2011, Bioinformation, Vol. 5(7), pp. 278-281.

8. *Rotary Culture Enhances Pre-osteoblast Aggregation and Mineralization.* **S.R. Facer, R.S. Zaharias, M.E. Andracki, J. Lafoon, S.K. Hunter, and G.B. Schneider.** 2005, Journal of Dental Research, Vol. 84(6), pp. 542-547.

9. *Tensile Mechanical Properties of Three-Dimensional Type I Collagen Extracellular Matrices with Varied Microstructure.* **Blayne A. Roeder, Klod Kokini, Jennifer E. Sturgis, J. Paul Robinson, Sherry L. Voytik-Harbin.** 2002, ASME, Vol. 124, pp. 214-222.